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Stedman's Medical Dictionary 27th Edition

neovascularization (ne'o-vas'ku-lar-i-za'shun)

Proliferation of blood vessels in tissue not normally containing them, or proliferation of blood vessels of a different kind than usual in tissue. **choroidal n.** ingrowth of new vessels from the choriocapillaris into the subretinal pigment epithelium and the retina; space associated with damage to the outer retina. **classic choroidal n.** well-demarcated areas of hyperfluorescence observed in the early phases of a retinal angiogram. **occult choroidal n.** area of leakage of undetermined source seen in the late phases of a retinal angiogram. **Type 1 choroidal n.** ingrowth of new vessels from the choriocapillaris into the subretinal pigment epithelial space; associated with damage to the outer retina. **Type 2 choroidal n.** ingrowth of new vessels from the choriocapillaris into the subretinal space; associated with damage to the outer retina.

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LYSINE BINDING FRAGMENTS OF ANGIOSTATIN

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority from provisional U.S. application Ser. No. 60/092,831, filed Jul. 14, 1998, incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention concerns peptide fragments of angiostatin containing lysine-binding sites of angiostatin, including small molecules that mimic their functions, which can be used as anti-angiogenic agents for the treatment of cancer, diabetic retinopathy, rheumatoid arthritis, psoriasis, atherosclerotic plaque formation, and any disease process that involves angiogenesis.

BACKGROUND OF THE INVENTION

Different than de novo vascularization, angiogenesis is the process of neovascularization from pre-existing blood vessels. It has pronounced effects in a wide array of physiological conditions, such as placenta development and embryogenesis. An imbalance of the angiogenic process has been shown to contribute to pathological disorders such as diabetic retinopathy, rheumatoid arthritis, and psoriasis (Folkman, J. (1995) *Nat. Med.* 1, 27-31; Folkman, J. (1995) *New Engl. J. Med.* 333, 1757-1763). Particularly, both primary and metastatic tumors need to recruit neovessels for their growth expansion (Folkman, J. (1971) *New Engl. J. Med.* 285, 1182-1186; Folkman, J., and Shing, Y. (1992) *J. Biol. Chem.* 267, 10931-10934).

There is a mounting body of evidence supporting the concept that angiogenesis is a prerequisite for tumor growth. Such a correlation has been established by blocking positive regulators of angiogenesis or utilizing negative regulators to suppress angiogenesis that resulted in a delay or regression of experimental tumors. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are potent mitogens and strong chemoattractants for endothelial cells (Shing Y., Folkman J., Sullivan, R., Butterfield, C., Murray, J., Klagsbrun, M. (1984) *Science* 223, 1296-1298; Maciag, T., Mehlman, T., Friesel, R., and Schreiber, A. (1984) *Science* 225, 932-935; Ferrara, N. and Henzel, W. J. (1989) *Biochem. Biophys. Res. Commun.* 161, 851-855). Their roles in inducing tumor angiogenesis have been demonstrated in a variety of human tumors (Nguyen, M., Watanabe, H., Budson, A. E., Richie, J. P., Hayes, D. F., and Folkman, J. (1994) *J. Natl. Cancer Inst.* 86, 356-361; Dvorak, H. F., Sioussat, T. M., Brown, L. F., Berse, B., Nagy, J. A., Sotrel, A., Manseau, E. J., Van de Water, L., and Senger, D. R. (1991) *J. Exp. Med.* 174, 1275-1278). Antibodies neutralizing VEGF or FGF caused a marked decrease of tumor growth via angiogenic inhibition (Gross, J. L., Herblin, W. F., Dusak, B. A., Czerniak, P., Diamond, M. D., Sun, T., Eidsvoog, K., Dexter, D. L., and Yayon, A. (1993) *J. Natl. Cancer Inst.* 85(2), 121-131; Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S., and Ferrara, N. (1993) *Nature* 362(6243), 841-844). Such anti-angiogenic and anti-tumor effects were also observed by antagonizing the corresponding receptors for these endothelial cell mitogens. A soluble form of Tie-2 receptor, a receptor tyrosine kinase preferentially expressed on vascular endothelium, exhibited effective inhibition of tumor angiogenesis (Lin, P., Polverini, P., Dewhirst, M., Shan, S., Rao, P. S., and Peters, K. (1997) *J. Clin. Invest.* 100(8), 2072-2078). Similarly, a

dominant negative mutant of the VEGF receptor, Flk-1, prevented VEGF-induced angiogenesis by dimerizing and deactivating the endogenous Flk-1 (Millauer, B., Shawver, L. K., Plate, K. H., Risau, W., and Ullrich, A. (1994) *Nature* 367(6463), 576-579). In addition, negative regulators of angiogenesis, such as angiostatin, endostatin, and antagonists for integrin $\alpha v \beta 3$, displayed profound anti-tumor activities in vivo (O'Reilly, M. S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R. A., Moses, M., Lane, W. S., Cao, Y., Sage, E. H., and Folkman, J. (1994) *Cell* 79, 315-328; O'Reilly, M. S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W. S., Flynn, E., Birkhead, J. R., Olsen, B. R., and Folkman, J. (1997) *Cell* 88(2), 277-285; Brooks, P. C., Stromblad, S., Klemke, R., Visscher, D., Sarkar, F. H., and Cheresch, D. A. (1995) *J. Clin. Invest.* 96(4), 1815-1822). TNP-470 and Interferon α -2a also manifested clinical evidence that tumor growth could be therapeutically intervened using an anti-angiogenic approach (Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., and Folkman, J. (1990) *Nature* 348, 555-557; Ezekowitz, R. A., Mulliken, J. B., and Folkman, J. (1992) *N. Engl. J. Med.* 326, 1456-1463).

Angiostatin was initially isolated from urine and sera of mice bearing Lewis Lung carcinoma (O'Reilly, M. S., et al., (1994) *Cell*; U.S. Pat. No. 5,639,725). It is an approximately 38-45kD internal fragment of plasminogen, which consists of 4 triple-disulfide bridged kringle structures. Both kringle 1 and 4 have lysine-binding sites, which are responsible for anchoring the plasminogen molecule on fibrin-rich blood clots (Wiman, B. and Collen, D. (1978) *Nature* 272, 549-545). Angiostatin was originally generated from plasminogen by proteolytic cleavage with porcine pancreatic elastase (O'Reilly, M. S., et al., (1994) *Cell*). Subsequent studies have shown that angiostatin can be generated from plasminogen by a variety of physiological and pathological proteases, including macrophage-derived metalloelastases (Dong, Z., Kumar, R., Yang, X., Fidler, I. J. (1997) *Cell* 88(6), 801-810), members of matrix metalloproteinase (MMP) family, such as matrilysin (MMP-7) or gelatinase B/type IV collagenase (MMP-9) (Patterson, B. C. and Sang, Q. A. (1997) *J. Biol. Chem.* 272(46), 28823-28825), and Urokinase (Gately, S., Twardowski, P., Stack, M. S., Cundiff, D. L., Grella, D., Castellino, F. J., Enghild, J., Kwaan, H. C., Lee, F., Kramer, R. A., Volpert, O., Bouck, N., and Soff, G. A. (1997) *Proc. Natl. Acad. Sci. USA* 94(20), 10868-10872). Angiostatin was found to inhibit endothelial cell proliferation in vitro and block growth factor, such as basic FGF, elicited angiogenesis in vivo. Elastase-cleaved angiostatin was shown to induce dormancy of several metastatic and primary tumors, including carcinomas of breast, prostate, colon, and lung (O'Reilly, M. S., et al., (1994) *Cell*; O'Reilly, M. S., Holmgren, L., Chen, C., and Folkman, J. (1996) *Nat. Med.* 2(6), 689-692). Angiostatin generated by urokinase cleavage significantly reduced the growth of a murine hemangioendothelioma in vivo (Lannutti, B. J., Gately, S. T., Quevedo, M. E., Soff, G. A., and Paller, A. S. (1997) *Cancer Res.* 57, 5277-5280). Recombinant angiostatin also produced anti-tumor effects in vivo via the blocking of tumor angiogenesis (Wu, Z., O'Reilly, M. S., Folkman, J., and Shing, Y. (1997) *Biochem. Biophys. Res. Commun.* 236, 651-654; Sim, B. K., O'Reilly, M. S., Liang, H., Fortier, A. H., He, W., Madsen, J. W., Lapcevich, R., and Nacy, C. A. (1997) *Cancer Res.* 57(7), 1329-1334). These anti-tumor effects were accompanied by a marked reduction of microvessel density within the tumor mass, indicating that suppression of angiogenesis led to the inhibition of tumor growth.

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CYCLIC-AMP AND CYCLIC-GMP PRODUCTION IN MCF-7 CELLS EXPOSED TO
ESTRADIOL-17 BETA, CATECHOLESTROGENS AND METHOXY-ESTROGENS IN MCF-7 CELLS
(MEETING ABSTRACT)

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Therapy of Breast Cancer. September 25-27, 1989, Genoa, Italy, A58, 1989.

Languages: ENGLISH

Document Type: MEETING ABSTRACT

The control of protein phosphorylation linked to cell-proliferating processes is unresolved. Cyclic-AMP (c-AMP) has been implicated in the inhibition of cell division; recent work, however, indicates that c-AMP enhances cell proliferation in many cell-types. The role of c-GMP is also not clear. It is known that estradiol-17 beta (E2) increases c-GMP levels and guanylate cyclase activity. Less is known of the effects of E2 on c-AMP production. We have previously shown that E2, the 2-/4-hydroxy E2 (2-OHE2; 4-OHE2), and the 2-methoxy E2 (2-MeOE2) metabolites affect cell division in MCF-7 cells. In this study, we determined the effects of E2, 2-/4-OHE2, and 2-/4-MeOE2 at dosages of 1×10^{-8} M on c-AMP and c-GMP production in MCF-7 cells growing in phenol red-containing (+PR) or in phenol red-free (-PR) MEM after 2-, 8-, 16-, and 24-hr exposure. Results were expressed as c-AMP or c-GMP/protein as a percentage of a 100% control. The most outstanding effect on c-AMP production in MCF-7 cells (+PR) was inhibitory and was seen 8 hr after exposure; E2 (71%), 2-OHE2 (75%), 4-OHE2 (78%), 2-MeOE2 (76%), whereas 4-MeOE2 had no effect. In MCF-7 cells (-PR), the greatest effect on c-AMP was a stimulation and was seen after 16-hr exposure to 4-MeOE2 (242%) greater than E2 (282%) greater than 4-OHE2 (189%) greater than 2-OHE2 (179%) greater than 2-MeOE2 (169%). Apparently, PR in MEM inhibited estrogen-dependent c-AMP production in MCF-7 cells. Significant stimulation of c-GMP production in MCF-7 cells (+PR) was seen after 8-hr exposure to 4-OHE2 (270%) greater than 4-MeOE2 (262%) greater than 2-MeOE2 (203%); E2 and 2-OHE2 had no significant effect; significant inhibition of c-GMP production was seen after 16-hr exposure to 2-MeOE2 (13.5%) greater than 2-OHE2 (13.5%) greater than 4-OHE2 (20.4%) greater than 4-MeOE2 (29%); E2 had no significant effect. In MCF-7 cells (-PR), only slight stimulation of c-GMP production was seen; after 8 hr only 4-MeOE2 (144%) and 2-MeOE2 (129%) increased the level and at 16 hr only 4-MeOE2 (128%). Apparently, PR in MEM increased c-GMP production in the presence of E2 and E2-metabolites. E2, 2-/4-OHE2, and 2-/4-MeOE2 failed to stimulate cell growth in MCF-7 cells (+PR). There is a positive correlation ($r = 0.92$) between E2-metabolite-induced decreased c-AMP levels at 8 hr and E2-metabolite-induced cell growth after 8 days (medium containing PR and the estrogens [1×10^{-8}] M) was changed every day). E2 and 2-/4-OHE2, but not 2-/4-MeOE2, increased cell growth in MCF-7 cells (-PR). A positive correlation ($r = 0.99$) between E2-metabolite-induced increases in c-AMP at 16 hr and E2-metabolite-induced cell growth on day 9 was observed (medium without PR but with E2 and E2 metabolites was changed every day).

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